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DOI:

[10.1016/j.foodhyd.2018.04.024](https://doi.org/10.1016/j.foodhyd.2018.04.024)

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*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Cassanelli, M, Prosapio, V, Norton, I & Mills, T 2018, 'Acidified/basified gellan gum gels: The role of the structure in drying/rehydration mechanisms', *Food Hydrocolloids*, vol. 82, pp. 346-354.  
<https://doi.org/10.1016/j.foodhyd.2018.04.024>

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Published in Food Hydrocolloids on 13/04/2018

DOI: 10.1016/j.foodhyd.2018.04.024

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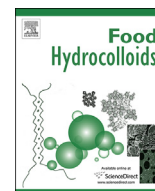
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# Acidified/basified gellan gum gels: The role of the structure in drying/rehydration mechanisms

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## ARTICLE INFO

### Article history:

Received 28 November 2017

Received in revised form

11 April 2018

Accepted 12 April 2018

Available online 13 April 2018

### Keywords:

Gellan gum

Mechanical properties

Acid/basic gelation

Gel drying/rehydration

## ABSTRACT

This work investigates the influence of pH variation on gellan gum gel structure upon drying and rehydration.

A comparison of the texture of wet gels prepared in acid and basic conditions showed that decreasing the pH of the gel to the pKa resulted in an increase in the gel strength and the Young's modulus. On further lowering the pH, both properties decrease dramatically. Post-gelation exposure to water and basic solution led to a reduction in mechanical properties, whereas exposure in acidic conditions resulted in gels with increased strength. The effect of the polymer concentration was investigated at pH 3.5 (corresponding to the highest strength and elastic modulus); it was observed that the gels set, even at very low concentrations, and that 0.5% w/w generated a gel as strong as the gel 2% w/w at natural pH. Thermal (DSC) and structural (FT-IR) analyses showed that when decreasing the pH down to the pKa, the polymer chains within the junction zones interact more strongly to each other and the structure has fewer disordered chains; below the pKa, the network became more aggregated and phase separation between the solvent and polymer occurred.

For all the gels prepared at different pHs, drying (air drying and freeze drying) and rehydration experiments were performed to investigate the water desorption/absorption rate in relationship to the gel structure. It was observed that the drying kinetics is not pH-sensitive. For both the drying techniques, it was observed that gels prepared at pH 4, recovered their initial shape, leading to higher rehydration ability.

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## 1. Introduction

Gels have a wide range of applications in the food industry (Banerjee & Bhattacharya, 2012), often being used as additives or gelling agents in the product formulation to design properties such as stability and thickness (I. Norton & Foster, 2002; Renard, van de Velde, & Visschers, 2006). Hydrocolloids are extensively used in the pharmaceutical and biomedical fields (Caló & Khutoryanskiy, 2015; Maiti, Ghosh, Mondol, Ray, & Sa, 2012).

One of the polymers frequently used in food and biomedical industries to prepare gels is low-acyl (LA) gellan gum, as it is non-toxic, biocompatible and biodegradable (Mahajan & Gattani, 2009). The native gellan gum form, or high-acyl (HA), is secreted as a linear extracellular anionic polysaccharide by the bacteria *Sphingomonas elodea* during aerobic fermentation and it is formed by a

tetrasaccharide repeating sequence consisting of two residues of  $\beta$ -D-glucose, one of  $\beta$ -D-glucuronate and one of  $\alpha$ -L-rhamnose (Fasolin, Picone, Santana, & Cunha, 2013; Morris, Nishinari, & Rinaudo, 2012). When dispersed in hot water, the polymeric chains are in the form of disordered single-coils. On cooling, the random coils initially rearrange to form threefold double helices (coil-helix transition), followed by aggregation of helices (sol-gel transition) on further temperature decrease, resulting in a three-dimensional gel network (Banerjee & Bhattacharya, 2012; Morris et al., 2012). On application of shear during gelation, the gel network domains become smaller, surrounded by a non-gelled continuous medium, forming a so-called "fluid gel" with spreadable and pourable properties (García, Alfaro, Calero, & Muñoz, 2011; A. B.; Norton, Hancocks, Spyropoulos, & Grover, 2016; I. T.; Norton, Frith, & Ablett, 2006; Sworn, Sanderson, & Gibson, 1995).

The rheological properties of gellan gum make it suitable for several applications (Bajaj, Survase, Saudagar, & Singhal, 2007; Prajapati, Jani, Zala, & Khutliwala, 2013; Stevens, Gilmore, Wallace,

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& in het Panhuis, 2016): in the food industry it is used as a stabiliser, or a thickening and gelling agent; in the pharmaceutical industry it is employed as carrier for controlled drug release; in cosmetics it is used as a stabiliser in shampoos, conditioners, creams, etc.; in biotechnology and biomedical engineering it is employed as a scaffold and medium for tissue cultivation.

In the food industry, the texture of the gel is a key parameter in order to design the food formulation and control the product characteristics (Bourne, 2002). One of the main parameters that affects the gel structure is the pH of the system: the attractive and repulsive forces between the polymer chains, as well as the interactions with the water molecules, change with it, resulting in the formation of different gel networks (Banerjee & Bhattacharya, 2012; A. B.; Norton, Cox, & Spyropoulos, 2011).

Picone and Cunha (Picone & Cunha, 2011) studied the effect of pH on the properties of gellan gum gels using rheological and calorimetric techniques: they found that an increase in pH resulted in a more fragile and deformable gel due to a decrease in molecular aggregation and an increase in the mobility of the strands. However, their experiments were restricted to limited pH conditions (3.5 for acid, 5.3 for neutral and 7 for basic). The gel properties at  $\text{pH} < 3.5$ ,  $3.5 < \text{pH} < 5.3$  and  $5.3 < \text{pH} < 7$  were not investigated, nor was the behaviour of gels exposed to different environments (acid and/or basic). Norton et al. (A. B. Norton et al., 2011) and Bradbeer, Hancocks, Spyropoulos, & Norton (2014) studied the textural properties of acidified gellan gum gels, observing that lowering the pH from 5 to 3 caused an increase in strength, whereas with a further reduction to pH 2, the structure became very weak, assuming a sponge-like behaviour. However, the gellan gum gels were not characterised from a molecular point of view.

If used as food additives (de Vries, 2002; Norton & Foster, 2002; Renard et al., 2006), gels may be subjected to drying to extend the product shelf-life (Mothibe, Zhang, Nsor-atindana, & Wang, 2011). Drying implies water removal to inhibit microorganisms' growth and enzyme activity, but can seriously damage the food microstructure with consequent change of properties, especially the texture (de Bruijn & Bórquez, 2014; Prosapio & Norton, 2017; Vega-Gálvez et al., 2015). Among the drying processes proposed in literature, air-drying and freeze-drying are the most commonly used. Air drying involves moisture removal by evaporation; it is a very simple technique, but it may cause several adverse effects on food attributes such as case hardening, shrinkage, poor rehydration and the alteration of colour, flavour and texture (Maskan, 2000; Nijhuis et al., 1998; Ratti, 2001). Freeze drying consists of the freezing of the product followed by water removal through sublimation at reduced pressure; it better preserves the food structure, but is characterised by long processing time and high energy consumption (Karam, Petit, Zimmer, Baudelaire Djantou, & Scher, 2016; Reyes, Mahn, & Cares, 2015). Drying of gellan gum gels has been studied by some authors (Bonifacio, Gentile, Ferreira, Cometa, & De Giglio, 2017; Chang, Im, Lee, & Cho, 2017; Czurzyńska, Marzec, Mieszkowska, & Lenart, 2017; Evageliou & Saliari, 2017; Silva-Correia et al., 2011; Tiwari, Chakkaravarthi, & Bhattacharya, 2015), but the effect of the gel pH on the drying and rehydration kinetics has not yet been investigated.

The aim of the present work is to investigate the behaviour of the gels prepared, not only in acid conditions, but also in a basic environment, providing a chemical interpretation of the mechanisms involved. The textural properties of the acidified/basicified gels will be studied after acid or basic exposure in order to provide a method for the design of the gel mechanics and, therefore, the food properties. The molecular interpretation of the pH effect on the gel structure is carried out by both thermal (DSC) and structural (FT-IR) analyses. In addition, for each pH, the effect of the drying technique on the gel microstructure will be evaluated, with a study of the

drying and rehydration kinetics of oven and freeze-dried samples. The textural properties of the rehydrated gels will also be investigated.

## 2. Material and methods

### 2.1. Materials

Low acyl gellan gum powder (Kelcogel F) was provided by CPKelco (UK). Citric acid (purity 99%) and sodium hydroxide (purity 98%) were supplied by Sigma-Aldrich (UK). All materials were used as received.

### 2.2. Gel preparation

Firstly, a solution of 2% (w/w) was prepared by dissolving the gellan powder in distilled water and stirring at 85 °C for 2 h to ensure a complete powder hydration and dissolution. The pH of the solution was measured using a pH meter (Seven compact, Mettler Toledo, UK) and was found to be equal to 5.2. For the acidified gels, the pH of the solution was adjusted by adding citric acid 0.3 M at the same temperature, whereas for basified gels sodium hydroxide (NaOH) 0.25 M was added. The solution was then placed into cylindrical moulds and, after gelation, the samples were 1 cm high with a 2.2 cm diameter.

### 2.3. Zeta potential

Zeta potential of gellan gum was measured in a Zetasizer Nano-ZS (Malvern Instrument, UK) using the acid-base titration method (Quast, 2016). Solutions of gellan gum were prepared with a concentration equal to 0.1% (w/w) to avoid gelation, yet a sufficient polymer concentration to perform the analysis. The pH of the solution (from 2 to 10.5) was adjusted by adding 0.25 M HCl or NaOH 0.25 M. For each solution, the electrophoretic mobility was measured at 25 °C and plotted as a function of the pH in order to experimentally calculate the pKa of gellan gum (Quast, 2016).

### 2.4. Mechanical properties

Mechanical properties were determined by uniaxial compression test using a texture analyser (TA.XT plus, Stable Micro System Ltd, UK), fitted with a 40 mm diameter cylinder probe, lubricated with silicon oil to minimise friction between the samples and the probe. The analyses were carried out with a compression rate of 2 mm/s. For each sample, the gel strength and elasticity were determined by measuring the resistance to 50% compression and the Young's modulus. Analyses were performed in triplicate.

### 2.5. Post-gelation exposure

All the gels prepared at different pH were placed in different environments for 24 h at 20 °C: acid medium at pH 2.5 formed by 0.3 M citric acid dissolved in distilled water; neutral medium formed by distilled water; basic medium at pH 11.5 formed by NaOH 0.25 M dissolved in distilled water. Later in the process, the samples were characterised again to highlight possible changes in the gel structure. Experiments were performed in triplicate.

### 2.6. Thermal behaviour

The thermal behaviour of the gels prepared at different pH was measured using a micro DSC 3 evo (Setaram Instrumentation, UK). Samples were accurately weighed ( $0.6 \pm 0.1$  g) and placed in the closed batch cell. Reference cell was filled with an equal mass of

distilled water. All the experiments were carried out from 10 °C to 90 °C with a scan rate of 1 °C min<sup>-1</sup>, applying a single heating/cooling cycle. In order to protect the system from the thermal history effect, isothermal periods were performed. The  $\mu$ DSC curves were plotted as an average of the cycles in triplicate and offset on the y-axis.

## 2.7. Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectra were obtained via the Spectrum Two IR Spectrometer (Perkin Elmer, UK) in reflection mode at a resolution of 4 cm<sup>-1</sup>. The scan wavenumber range was 4000–600 cm<sup>-1</sup>, and 16 scan signals were averaged to reduce the noise.

## 2.8. Oven drying

Conventional drying tests were carried out introducing the wet gels in an oven (Fistreem International Co. Ltd, UK) with no flow air, at 40, 50 and 60 °C, room pressure and a constant relative humidity (RH) about 20%. Experiments were performed in triplicate.

## 2.9. Freeze drying

Wet samples were frozen at –20 °C and then lyophilised using a bench top Freeze Dryer (SCANVAC Coolsafe™, model 110-4, Denmark), condenser temperature –110 °C, pressure 10 Pa, condition that is defined by the equipment. Experiments were performed in triplicate.

## 2.10. Moisture content analysis

Moisture content (MC) analyses were carried out measuring the sample weight at regular intervals.

Moisture content was expressed as NMC (Normalised Moisture Content) (Brown, Fryer, Norton, & Bridson, 2010) through the following equation (1):

$$NMC = \frac{(M_d - M_s)}{(M_o - M_s)} \quad (1)$$

where  $M_s$  is the solid sample mass, calculated by using the constituent mass percentage used for the gel preparation,  $M_d$  the sample mass after drying, and  $M_o$  the sample mass before drying. The drying kinetics was determined plotting NMC as a function of the drying time.

The content of citric acid present in each gel (added to adjust the pH) was calculated subtracting the weight of the polymer (2% w/w) and the weight of the water from the total weight of the gel. The water amount was determined using a moisture analyser (model MB 25, OHAUS, Nanikon, Switzerland). Two grams of sample were placed within the aluminium pans and located over the pan support of moisture meter. A halogen element inside the moisture meter provides uniform infrared heating. It heats the sample at a set temperature of 120 °C until the sample weight becomes constant. Moisture percentage as a function of weight change was recorded and displayed. Analyses were performed in triplicate.

## 2.11. Porosity

Dried gels were analysed by X-ray micro-computed tomography using the Skyscan 1172 (Bruker, Belgium). The acquisition mode was set at a maximum current of 96  $\mu$ A and voltage of 100 kV. The CT-analyser (1.7.0.0) was used to obtain both a qualitative and quantitative analysis. After 2D cross-section binarisation into black

and white images, the porosity on the whole bulk structure and the pore size distribution was measured.

## 2.12. Rehydration

Rehydration experiments were performed by immersing a weighed amount of dried samples into distilled water at room temperature. The samples were removed at regular intervals, blotted with paper to eliminate the surface water and then reweighed. NMC was measured using equation (2):

$$NMC = \frac{(M(t) - M_s)}{(M_o - M_s)} \quad (2)$$

where  $M(t)$  is the sample weight at time  $t$ ,  $M_s$  is the solid sample mass, and  $M_o$  the pre-dried sample. Then, the rehydration behaviour was determined plotting NMC as a function of the time.

At regular intervals the solid content was measured by using the moisture analyser. It was observed that the evolution of NMC, due to the solubilisation of citric acid in water, was less than 1%.

# 3. Results and discussion

## 3.1. Effect of pH on gel structure and mechanics

The investigation of the pH effect on the mechanical and physical properties of LA gellan gum gels was carried out by texture analysis. In Fig. 1A–B the gel strength and the Young's modulus are reported as a function of the gel pH.

It is shown that increasing the H<sup>+</sup> concentration of the hot solution by citric acid addition the gel strength to reach a 50% strain on compression initially rises up to the maximum of 87.7 N  $\pm$  5.0 N at pH 3.5. A further decrease in pH leads to a considerable drop in strength with an evident loss in transparency (Bradbeer et al., 2014), and a sponge-like behaviour (A. B. Norton et al., 2011). A similar trend was observed for the elastic modulus with the highest gel stiffness at pH 3.5. All the compressed gels failed after a 50% deformation. The basified gels showed a textural behaviour similar to the gel in natural conditions, since a difference less than 5 N and 2 kPa was observed for the gel strength and elastic modulus, respectively.

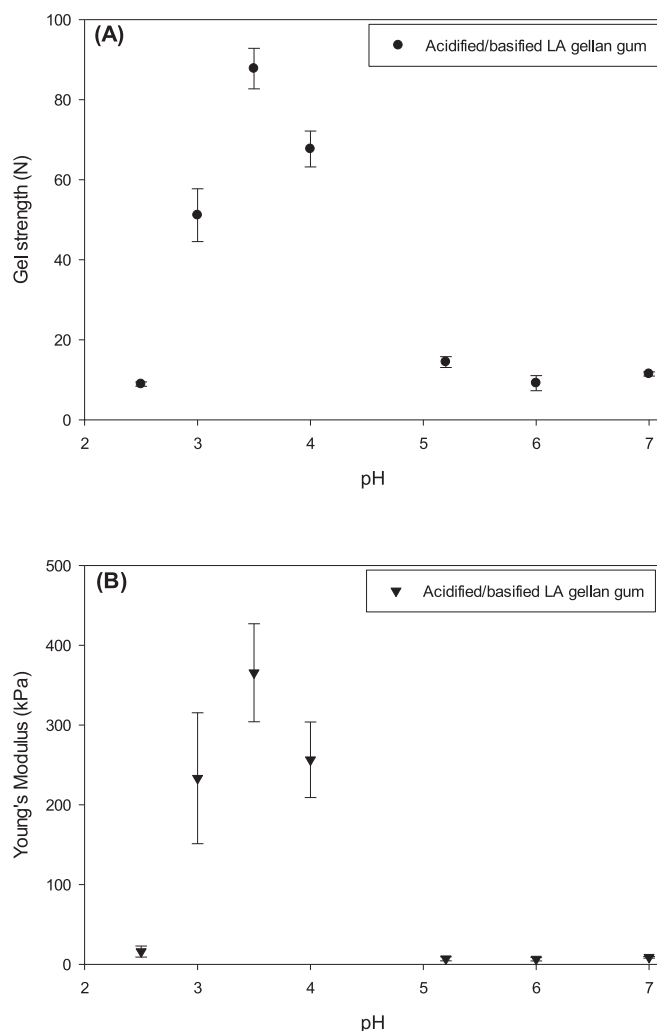
In agreement with A. B. Norton et al. (2011), the gels that require the highest force were the ones produced at the pH around the gellan gum pKa. Fasolin et al. (2013) reported a pKa around 3.5 for low-acyl gellan gum (Kelcogel®F) by measuring the zeta potential as a function of pH. This value is in agreement with the experimental pKa, obtained by measuring the electrophoretic mobility. This method follows the procedure proposed by Mehrishi and Seaman (1968); Quast (2016): at high pH the mobility tends to reach a plateau because the polymer chain might be fully ionised. On pH reduction, the polymer becomes less negatively charged. The pKa is considered as being the specific pH at which the electrophoretic mobility is half of the plateau value.

The reason for the increase in the gel strength by pH reduction down to 3.5, followed by the decrease in mechanical properties on further pH reduction (Fig. 1A–B), might be related to the negative charge percentage on the polymer chains. The Henderson-Hasselbach equation (eq. (3)) correlates the solution pH and the compound pKa and shows that the ionisable solute is half dissociated for pH = pKa.

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad (3)$$

where [HA] is the molar concentration of the weak acid and [A<sup>-</sup>]





**Fig. 1.** Gel strength (A) and elastic modulus (B) as a function of LA gellan gum (2% w/w) gel pH.

the molarity of the conjugate base of the acid. From this equation, it is deduced that the highest dissociation is for pH higher than the  $pK_a$ . In other words, the gellan gum system evolves as a function of the pH, not only in terms of available cations, but also in terms of polymer ionisation.

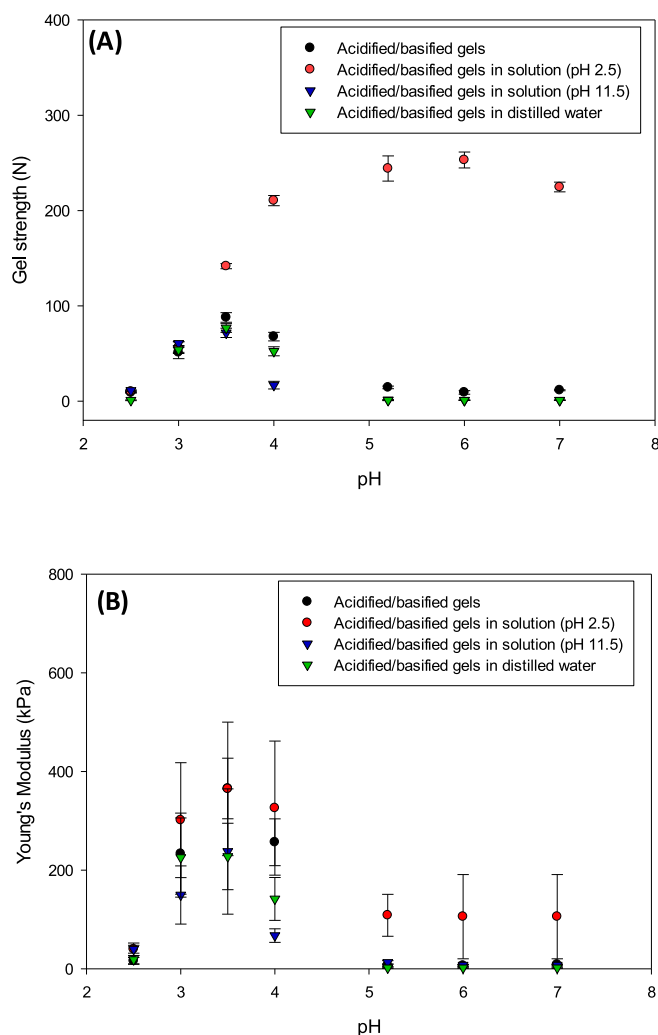
During the gel preparation, the hot solutions at natural or higher pH contain highly ionised polymer chains. In effect, the gellan gum zeta potential at natural pH (5.2) is equal to  $-20.3 \text{ mV} \pm 1.1 \text{ mV}$ , whereas at pH 3.5 is  $-12.2 \text{ mV} \pm 0.8 \text{ mV}$ . In order to form the gel network, cations are required, interacting with the negative charges and forming aggregated regions, the so-called junction zones (Morris et al., 2012). In the Kelcogel<sup>®</sup>F formulation, both monovalent ( $\text{Na}^+$  and  $\text{K}^+$ ) and divalent ( $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ ) cations are present, added as chlorides up to around 5% w/w (Amici, Clark, Normand, & Johnson, 2000; CPKelco, 2007). The gel in this condition can set, if the gel concentration is above the critical one (Phillips & Williams, 2009), although it may result in a weak gel in terms of mechanics (A. B. Norton et al., 2011).

It has been observed that the linear charge density (Morris et al., 2012) of the polyanion decreases as the pH of the aqueous solution becomes lower (Rizwan et al., 2017), by protonation of the carboxylate groups (Bradbeer et al., 2014). This consideration is in agreement with the zeta potential and electrophoretic mobility results, which indicate that the overall polymer charge changes as a

function of the pH. The gel chains at natural pH have a high linear charge density, which in the presence of cations form junction zones. The dimensions of these highly ordered domains do not cause scattering, as the gels are transparent (A. B. Norton et al., 2011). When the pH is decreased, the linear charge density on the gellan gum molecules is reduced, whereas the amount of cations available in the medium is raised by the acid addition. The junction zones reduce in number, yet the interaction between the chains increases due to the presence of more cations. However, when the pH equates the  $pK_a$  or it is below this value, the polymer becomes nearly neutral, as the cations (provided by salt addition in the initial formulation or upon acidification) can balance the negative charge of the carboxylate groups of the polymer. Moreover, the excess of positive charges may induce local chain repulsion and further weaken the gel network. As the gel is more turbid for low pHs, this suggests that the polymer is more aggregated, with packed domains of gellan gum chains. The low electrostatic interaction leads to phase separation between the polymer and solvent (Bradbeer et al., 2014; Morris et al., 2012). This can explain why the gel assumes a sponge-like behaviour with a low capability to hold water, especially under stress (A. B. Norton et al., 2011). Similar behaviour but due to a different principle is the sponge-like structure of cryogels (Kumar, Mishra, Reinwald, & Bhat, 2010; Lozinsky et al., 2003). In these systems, water can more easily move through the large channels of the highly porous structure and can be removed during compression.

The trend of the mechanical properties for the acidified gels was preserved, even if they were submerged in distilled water (pH-neutral environment) for 24 h (Fig. 2A–B). Once the  $\text{H}^+$  cations electrostatically interacted with the polymer, it seems that they were minimally released from the structure, since the gel strength decreased only slightly. This reduction might be due to the moderate LA gellan gum gel swelling and subsequent network expansion, which made the structure softer, as Cassanelli, Norton, and Mills (2017) showed. The results obtained after acid/basic exposure (Fig. 2A–B) confirmed that the gel strength is strictly related to the presence of junction zones and the interaction of the chains within them, as the linear charge density may vary (Morris et al., 2012).

The acidified/basified gels were then placed in water with citric acid at pH 2.5 or NaOH at pH 11.5 for 24 h. For the acid solution exposure, it was evident that gels formed at relatively high pH could generate a considerably stronger structure, around 15 times the initial gel strength, while at low pH this effect was negligible. It is believed that this is related to the fact that the high-pH polymer is highly ionised and the subsequent addition of  $\text{H}^+$  leads to larger and more compacted junction zones by saturating the negative charges. It seems that the hydrogen ions interact with these available negative charges, after penetrating the structure. At the same time, the extent of disordered domains decreases, resulting in considerably stronger gels (Morris et al., 2012). Upon soaking the low-pH gels in the acid solution, this effect became less evident, as the initial negative charges were already reduced, as shown for pH 3 and 2.5. Since the gel strength at pH 3.5 slightly increased, it suggests that at this pH the polymer was not completely neutral and the negative charges were not yet saturated by cations. Similarly, the acid exposure affected the elastic properties of the gels, as the Young's modulus increased (Fig. 2B). Interestingly, none of the gels showed signs of failure during compression to 50% strain, yet they could not recover the initial height when the stress was removed. For the basic solution exposure, the gel strength and elastic modulus did not increase. The slight decrease in gel strength suggests that water might swell the structure, making it weaker, as well as the high  $\text{OH}^-$  concentration that might influence the junction zone size by neutralising cations.

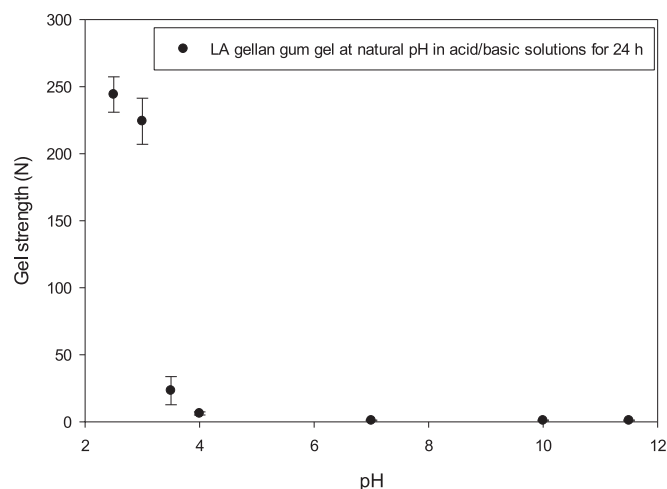


**Fig. 2.** Gel strength (A) and elastic modulus (B) as a function of LA gellan gum (2% w/w) gel pH and exposure to distilled water, acid (pH 2.5) or basic (pH 11.5) solutions for 24 h.

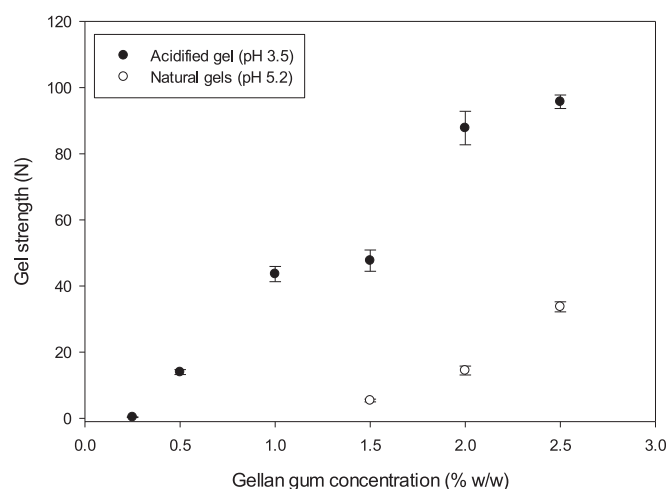
In order to investigate further the influence of the acid solution exposure, gels at natural pH (5.2) were treated for 24 h in solutions at different pH, as shown in Fig. 3. It seems that the amount of  $H^+$  required to interact with all the negative charges on the polymer is pH 3 or less. Above pH 3, the amount of cations provided is not sufficient to make the junction zones larger. In addition, when the gels were soaked in aqueous solution, a decrease in gel strength was observed at pH higher than 3, mainly due to the network expansion, as shown in Fig. 2A–B.

At pH 3.5 the effect of the polymer concentration on the gel strength was evaluated and compared to the natural-pH gel (Fig. 4). It was observed that at pH 3.5 a gel can set even at very low gellan gum concentration (0.25% w/w). Interestingly, 0.5% w/w generates a gel as strong as the 2% w/w gel at natural pH. On the other hand, the gel at the natural conditions at 0.5% w/w cannot form a firm sample with a cylindrical shape. This behaviour might be particularly beneficial for the food formulation design to achieve comparable mechanical properties with a reduced concentration in gelling agent.

The gels were further investigated at the molecular level with both  $\mu$ DSC and FT-IR techniques. In Fig. 5A–B the  $\mu$ DSC thermograms for the acidified/basified gels on cooling (A) and heating (B)



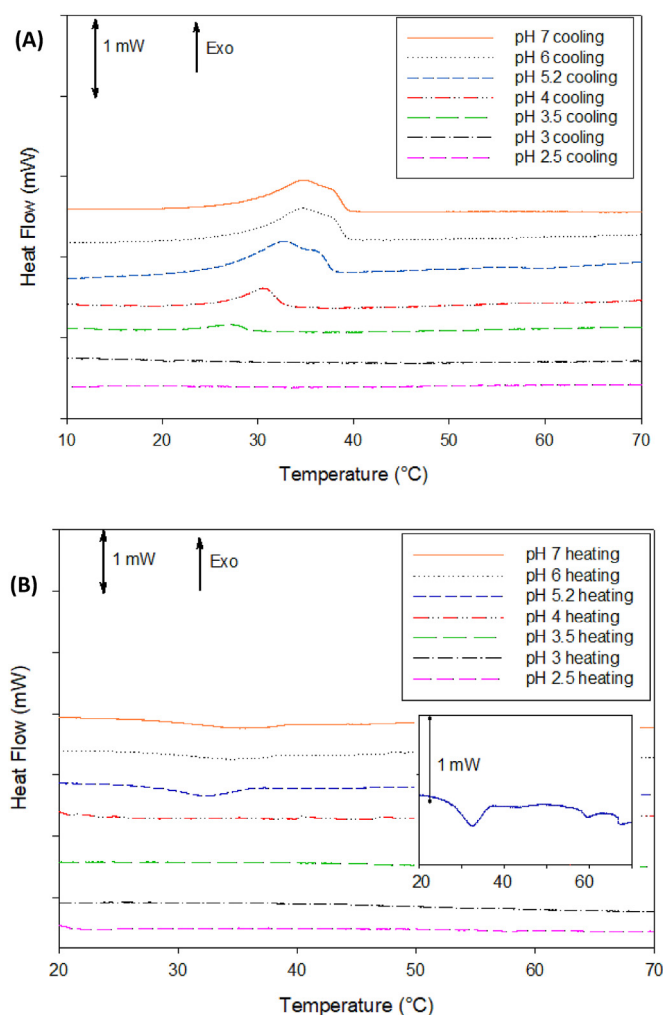
**Fig. 3.** Gel strength of 2% w/w LA gellan gum (natural pH) as a function of the exposure medium pH.



**Fig. 4.** Gel strength as a function of LA gellan gum concentration at natural pH (5.2) and pH 3.5.

are reported. At the natural pH (5.2) a single exothermic peak was noted (Fig. 5A), suggesting that the formation and aggregation of double helices take place at very close temperatures, as Picone & Cunha (2011) reported. It seems that two peaks are overlapping. On the other hand, the endothermic peak at  $\sim 30^\circ\text{C}$  is likely to be related to the melting of non-aggregated gellan gum helices (Fig. 5B), while other transitions at higher temperatures lead to the complete gel melting (Picone & Cunha, 2011; Robinson, Manning, & Morris, 1991).

The thermograms (Fig. 5A) for the basified gels (pH 7 and 6) are similar to the gel at pH 5.2, showing a single peak formed of the two exothermic transitions. On decreasing the pH, the related enthalpy was found to decrease (Table 1). It suggests that disordered chains associate, forming junction zones progressively smaller in number. Below the  $pK_a$  no peaks were evident on cooling, indicating that the gel did not melt in the experimental temperature range as it was highly aggregated. In agreement with the enthalpy reduction, the peak temperature shifted to lower values. It suggests that the disordered chains that undergo the coil-helix transition become progressively shorter and a lower temperature is required for the transition to occur. The acidified gels at pH lower than 4 do not have

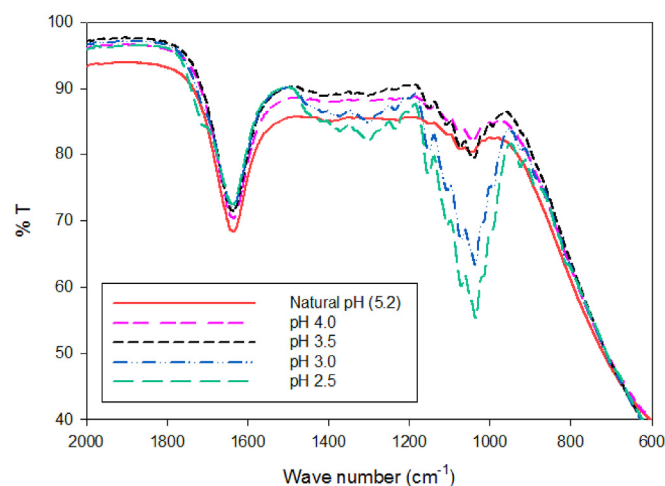


**Fig. 5.** mDSC thermograms for acidified/basified gels (2% w/w) on cooling (A) and heating (B). A magnification of the curve on heating related to gel at pH 5.2 is reported (B).

clear second transitions related to the melting of the junction zones (Picone & Cunha, 2011), as shown in Fig. 5B.

In Fig. 6 the infrared spectra for the acidified gels are shown. As the gellan gum chains interact more within the junction zones for the gels at pH 4 and 3.5, less intense bands in the wave number range between 1170 and 960  $\text{cm}^{-1}$  were observed compared with the gel at natural pH. In this range, the peaks are likely to be related to the CO stretching and the interactions with cations can limit this vibration band. On the other hand, on further pH decrease there are more intense peaks, suggesting that the polymer chains electrostatically interact to a lesser extent.

This work shows that the mechanical properties are highly affected if the gels were acidified/basified before gelation or after gel setting. If the acid/base is added into the hot solution, it is important to consider that the charges on the polymer change and, therefore, their interaction with the cations. On the other hand, if it



**Fig. 6.** FT-IR spectra for acidified gels (2% w/w).

is added after gelation, the gel might become considerably stronger if initially produced at pH above the pKa. As a food engineering application, the product stability and thickness might be controlled and tailored to the final application by modulation of pH.

### 3.2. Gel drying and rehydration

The gellan gum gels prepared in acid/basic conditions were studied in terms of drying and rehydration kinetics. Specifically, the samples were initially freeze-dried or oven-dried, followed by rehydration in distilled water up to 24 h. Fig. 7 shows the freeze-drying (A) and oven-drying (B) kinetics curves. As expected, the drying rate was considerably higher for the oven drying, since it is based on the evaporation at relatively high temperatures. From the microstructural point of view, no differences were observed within the oven-drying temperature range investigated. However, the pH did not affect the water removal rate, as the drying kinetics curves tended to overlap in both processes.

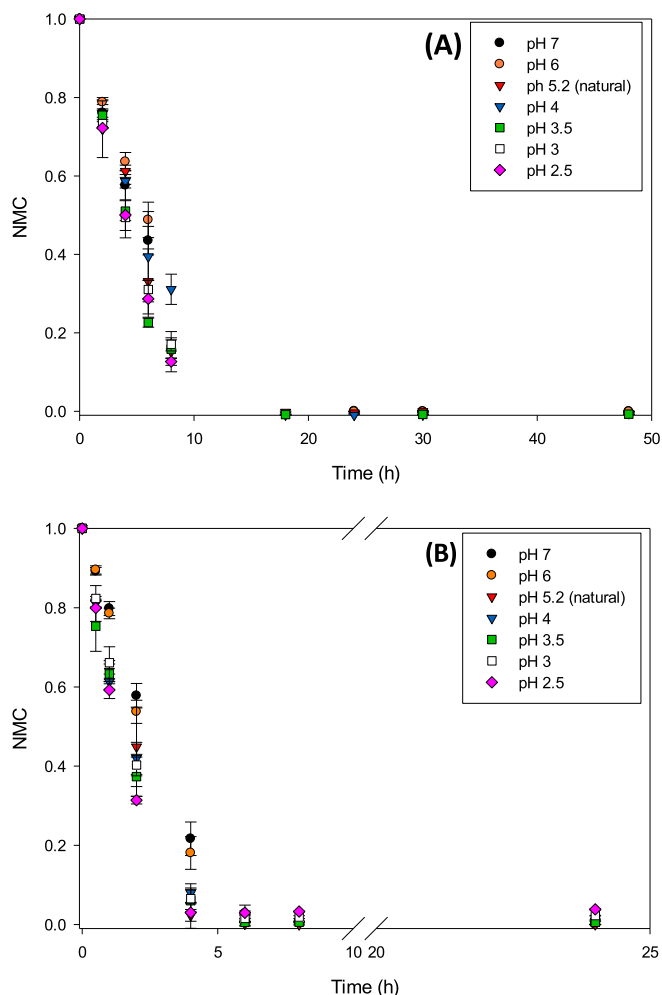
Contrary to the drying kinetics, the rehydration rate was influenced by the pH of the solution, as shown in Fig. 8 A–B. Overall, the freeze-dried gels show a quicker water uptake, especially for short timescales, while the oven-dried ones tend to rehydrate more slowly. The reason lies in the microstructural differences between the two sample types. Freeze-drying generates relatively large pores due to the formation of ice crystals during the freezing step and, therefore, a highly porous material (Rey, 2016). On the other hand, water removal through evaporation leads to the collapse of gel structure, increasing the material bulk density and thus making the rehydration process slower.

The pH affected the extent of rehydration and rehydration rate of both freeze-dried and oven-dried gels. Specifically, in Fig. 8A it is shown that, by decreasing the pH down to the pKa, the initial water uptake of freeze-dried samples decreases. It is likely to be related to the formation of smaller pores due to the citric acid addition during the gel preparation, which should enhance the ice crystal nucleation rate rather than their growth (Petzold & Aguilera, 2009). In effect, the total porosity, embedding both open (interconnected)

**Table 1**

Peak temperature and enthalpy of thermal transitions on cooling for LA gellan gum at 2% w/w as a function of gel pH.

	pH 7	pH 6	pH 5.2	pH 4	pH 3.5
Temperature (°C)	33.5 ± 0.9	33.9 ± 0.7	32.7 ± 0.1	30.9 ± 0.1	28.1 ± 0.6
ΔH (J g <sup>-1</sup> )	-0.216 ± 0.039	-0.238 ± 0.036	-0.200 ± 0.005	-0.124 ± 0.016	-0.054 ± 0.022

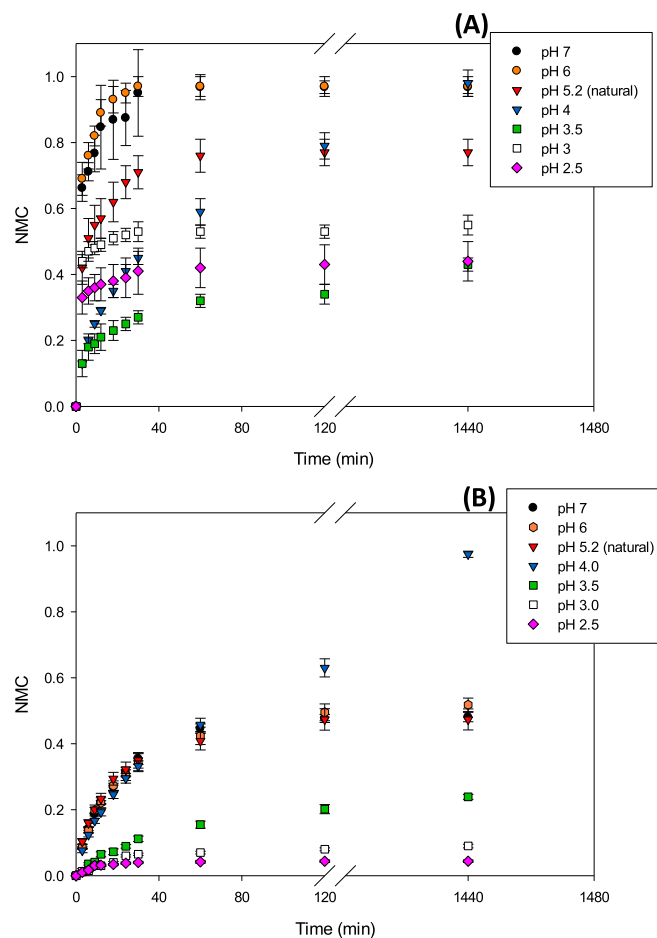


**Fig. 7.** Drying kinetics curves for 2% w/w LA gellan gum expressed as NMC over time: (A) freeze drying; (B) oven drying at 50 °C.

and closed (non-interconnected) pores, moved from  $84.8 \pm 4.2\%$  (natural pH) to  $55.2 \pm 5.2\%$  (pH 2.5). A more rigid material, due to the polymer chain aggregation, may influence more the crystal expansion, compared to a softer gel (Scherer, 1990). By further decreasing the gel pH (2.5 and 3), the initial water uptake became slightly higher. The curves related to pH 2.5 and 3 are close to each other, at slightly lower values for the former. As shown in Table 2, by decreasing the pH of the solution, the solid content of the gel increases, achieving about 4.1% at pH 3 and 8.5% at pH 2.5. As a consequence, a more aggregated and compacted material is obtained, which may obstruct the water uptake.

The oven-dried gels tended to rehydrate more slowly and to a smaller extent as the pH decreased (Fig. 8B). A rigid-collapsed network, generated by decreasing the pH to 3.5, might re-absorb less water over a longer timescale, since the more aggregated structure resists expansion of the gel. At low pH, the rehydration rate is even lower, although the structure is less aggregated. It might be related to the higher solid content percentage, which is likely to decrease the material porosity (from  $7.3 \pm 0.7\%$  at natural pH to  $4.6 \pm 3.3$  at pH 2.5) and increase the pore-wall thickness.

In terms of shape and volume retention as well as mechanical properties in comparison with the gels before drying, it is interesting to observe that the acidification at pH 4 led to a relatively higher appearance quality for both the drying methods (Fig. 9A–B–C–D). In effect, at the end of the 24-h rehydration in distilled water



**Fig. 8.** Rehydration curves for 2% w/w LA gellan gum expressed as NMC over time. The dried samples were placed in distilled water after freeze drying (A) and oven drying (B).

**Table 2**

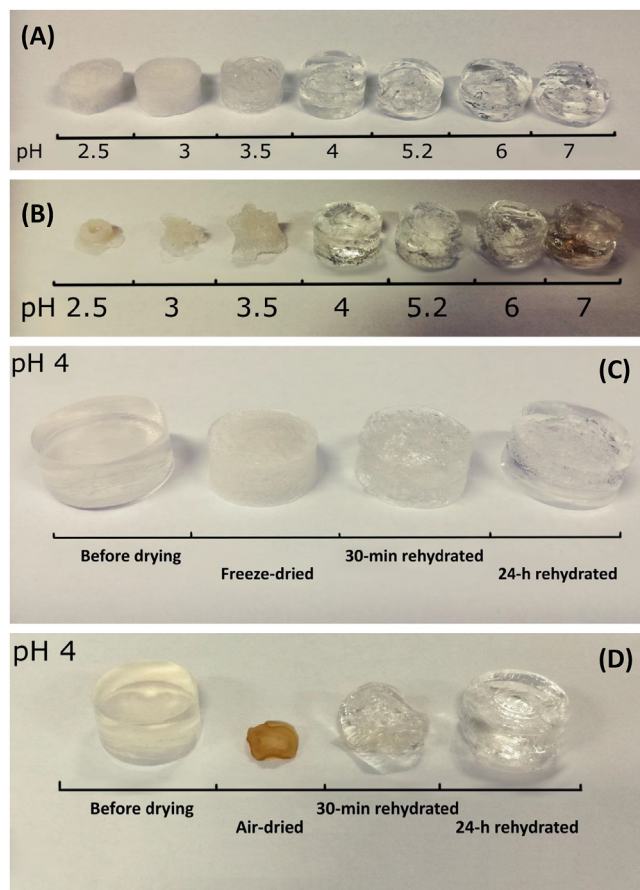
Water content percentage before drying for acidified LA gellan gum gels at 2% w/w. The gel pH is adjusted by adding citric acid.

pH	Water content %
4	$97.0 \pm 0.6$
3.5	$96.9 \pm 0.5$
3	$95.9 \pm 0.5$
2.5	$91.5 \pm 0.7$

the gels were visually similar to the ones before drying and an increased turbidity by decreasing the pH was observed after rehydration (Fig. 9A–B).

In Fig. 10A–B the mechanical properties for the rehydrated gels are reported in comparison with the gels before drying. Specifically, the freeze-dried and rehydrated gels at higher and lower pH than 4 became less strong. The freeze-dried and rehydrated gels at pH 3.5 show a drop in gel strength (Fig. 10A). Since the gel network is more rigid and stronger because of the junction zones formed at this pH, the ice crystals might be less accommodated. Below the pKa the gel did not recover the initial properties before freeze-drying, having a gel strength after 50% strain between 0 and 1 N. However, it is important to mention that the final mechanical properties are lower for each pH. In effect, although a polymer chain alignment occurs due to the ice crystal formation, the freeze-dried material

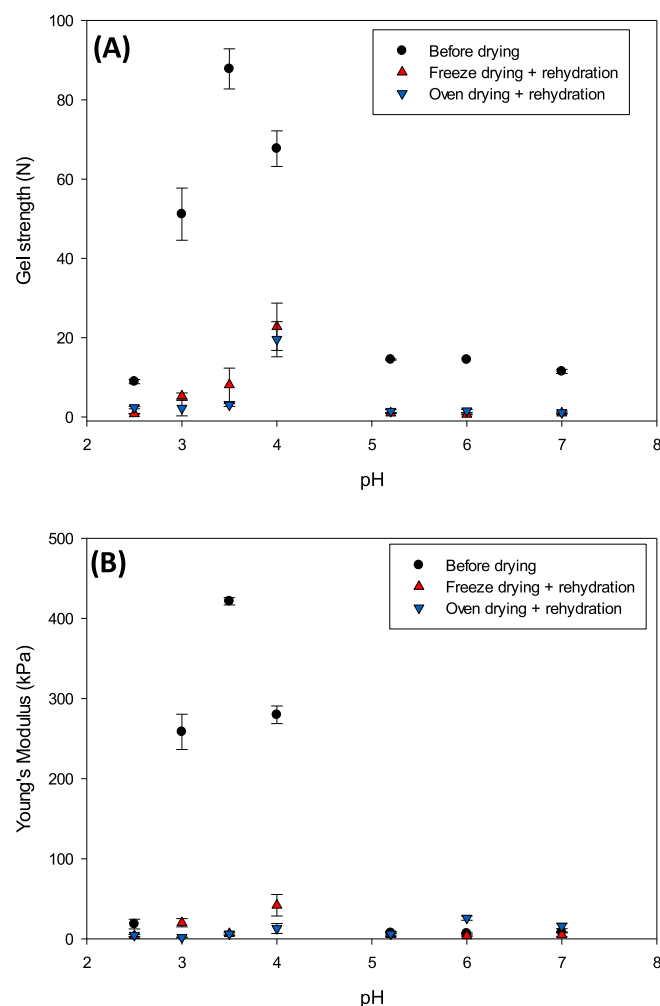




**Fig. 9.** 24-h rehydrated samples (2% w/w) after freeze-drying (A) and oven drying (C). Gel comparison (pH 4) on rehydration after freeze-drying (B) and oven drying (D).

has large pores that behave like cracks, resulting in a gel strength decrease, in addition to the effect of distilled water, as previously discussed (Fig. 2A–B). It seems that pH 4 generates the optimum junction zone formation, leading to an intermediate behaviour in terms of strength and elasticity, which appears to preserve more the mechanical properties after freeze-drying and rehydration. Similarly, the oven-dried and rehydrated gels showed that the gel mechanics and geometry were minimally affected in the case of the gels at pH 4. In this context, the gel pH decrease is necessary to enhance the polymer network rigidity and avoid that the gel become too soft after rehydration. However, if the aggregation level is further increased, the recovery of gel shape and mechanics is obstructed, similarly to the freeze-dried and rehydrated samples. Below the pKa the gel might start to have a more disordered structure which might make the gel shape/volume recovery more difficult (Fig. 9A–B). However, especially the gel at pH 2.5 did not recover the initial shape, influencing the texture measurement, as it was collapsed and compacted even after rehydration.

The freeze- and oven-dried gels at pH 4 were then rehydrated in both acid (pH 2.5) and basic (pH 11.5) solutions to evaluate the effect on the dried and rehydrated gel structure. For the freeze-dried samples, it was noted that the peak force reached  $7.40 \pm 1.76$  N, a value comparable to  $8.11 \pm 4.22$  N for the rehydration in distilled water. It seems that during the water uptake the gels did not become stronger. However, it is difficult to predict if larger and stronger junction zones are formed on rehydration in acid solution, since the system was highly affected by the presence of large pores formed after freeze-drying. In the basic solution, the gels became softer, showing a peak force equal to  $0.70 \pm 0.11$  N, due



**Fig. 10.** Gel strength (A) and elastic modulus (B) of dried and rehydrated gels (2% w/w) as a function of their pH.

to the effect of the high  $\text{OH}^-$  concentration, as discussed in section 3.1 about the gel exposure to the basic solution.

The initial cylindrical shape of the air-dried gels, lost after the structure collapse, was not recovered if an acid solution was used. It is likely to be related to the change in gel network throughout the rehydration. As a result, the gel strength was affected by the irregular shape and compacted material and was equal to  $1.94 \pm 0.18$  N. On the other hand, the effect of the basic solution generated rehydrated gels with a less defined shape as well as a lower gel strength ( $3.05 \pm 1.52$  N), similarly to the freeze-drying case.

Overall, it seems that the rehydration in a neutral environment is more suitable to recover the geometrical properties of the gel and have a relatively stronger material for the gels previously freeze- and oven-dried.

#### 4. Conclusions

It has been demonstrated that it is possible to modulate the mechanical properties of gellan gum gels by adjusting the pH of the solution during gelation and by acid/basic environment exposition. The gel structure aggregation and the formation of junction zones have been correlated to the electrostatic interactions and cation condensation that take place according to the gelation conditions. It results in changes in gel strength and in elastic modulus.

Both the freeze- and oven-drying of gels are not pH sensitive in terms of drying kinetics. However, the rate and the extent of the gel rehydration depends on the structure aggregation/junction zones and porosity and, therefore, on the gel pH. Regardless of the drying method, a drop in the rehydration rate was observed at pH close to the gellan pKa, in occurrence of the formation of larger and stronger junction zones.

This study can be particularly useful for the design of the food formulation and to predict possible changes in food additives when subjected to different environments and/or drying.

## Acknowledgment

This research was funded by the Engineering and Physical Sciences Research Council [grant number EP/K030957/1], the EPSRC Centre for Innovative Manufacturing in Food.

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